

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1. (currently amended): A method for ~~development of~~ developing nucleotide probes for myctophid fishes, said method comprising the steps of:

- (i) extracting ~~the~~ DNA from the muscle tissue of a myctophid fish,
- (ii) selecting a gene region in the extracted DNA as a DNA template and amplifying the selected gene region with ~~the a pair of forward and backward~~ selected primers ~~and the amplifying the same~~ using polymerase chain reaction (PCR),
- (iii) eluting the PCR amplified DNA containing the selected gene region,
- (iv) re-amplifying and re-eluting the ~~gene regions from PCR amplified DNA and eluting the same~~ amplified DNA in step (iii),
- (v) cycle sequencing the ~~of~~ eluted DNA containing the selected gene region using a single primer to produce an extension product,
- (vi) purifying the extension ~~products~~ product containing the selected gene region,
- (vii) sequencing the nucleotides of the extension product of step (vi) on an acrylamide gel,
- (viii) confirming the ~~sequences for the~~ nucleotide sequence of the selected gene region target gene by Blast-Email,
- (ix) ligating the ~~eluted PCR products in~~ extension product containing the selected gene region as a DNA insert into a cloning vector,
- (x) preparing ~~the electro-competent~~ host cells for electro-transformation,
- (xi) electro-transforming the host cells with the vector-containing DNA insert,
- (xii) growing and harvesting of transformed host cells ,
- (xiii) re-inoculating and growing transformed host cells that appear as white colonies and that express the DNA insert containing the selected gene region;
- (xiv) confirming the presence of the DNA insert containing the selected gene region that the transformed bacteria has the plasmids with the gene inserts by PCR, by polymerase chain reaction,

(xv) purifying ~~recombinant plasmid DNA having the cloned gene probes the~~ cloned DNA insert containing the selected gene region from the transformed host cells to produce a DNA probe,

(xvi) checking the purity and specificity of the ~~cloned DNA probe insert DNA~~ probe by cutting with a restriction enzyme,

(xvii) confirming the molecular size of the ~~DNA insert~~ DNA probe,

(xviii) ~~PCR amplification of the gene insert from the probe using both primers~~ amplifying the DNA probe using the selected set of forward and backward primers of step (ii),

(xix) eluting ~~of~~ the amplified gene region DNA probe containing the selected gene region,

(xx) cycle sequencing ~~of the gene region of the probe~~ the eluted DNA probe in step (xix) using a single set of primer,

(xxi) sequencing ~~of the cloned insert~~ eluted DNA probe in step (xix) on an acrylamide gel,

(xxii) comparing the nucleotide sequence of the ~~prepared DNA probes~~ DNA probe using "BLAST program" against the known sequences of similar genes in the genome data bases,

(xxiii) confirming the sequences sequence of the ~~cloned~~ DNA probe by aligning with ~~sequences of the claim 1~~ sequence obtained in step (vii), and

(xxiv) designing species specific primers ~~from the sequences~~ based on the sequence of the DNA probe.

Claim 2. (currently amended) A method ~~as claimed in~~ of claim 1, wherein the myctophid ~~fishes are~~ fish is selected from the group ~~comprising~~ consisting of *Stenobrachis leucopsarus*, *Diaphus theta*, *Protomyctophum crockeri*, *Tarletonbeania crenularis* and *Lampanyctus regalis*.

Claim 3. (currently amended) A method ~~as claimed in~~ of claim 1, wherein the gene ~~regions are~~ region is ~~selected from~~ a mitochondrial control gene region and or a nuclear gene region.

Claim 4. (currently amended) A method ~~claimed in~~ of claim ~~13~~, wherein the mitochondrial ~~genes taken for probe preparation are selected from the group~~

~~comprising:~~ control gene region is from a Cyt b<sub>2</sub> and a D-loop genes, a 12S RNA ~~and or~~ a 16S RNA ~~genes gene~~.

Claim 5. (withdrawn) A method claimed in claim 1 wherein the nuclear genes taken for probe preparation are selected from Rod and ITS-2 genes.

Claim 6. (withdrawn) A method of claim 1 wherein the PCR amplified cleaned nuclear gene probe is Rod gene.

Claim 7. (withdrawn) A method claimed in claim 1 wherein the nuclear gene taken for the cloned probe preparation is ITS-2 gene.

Claim 8. (currently amended) A method ~~as claimed in of~~ claim 1<sub>2</sub> wherein the concentration of the forward and backward primers ~~used for PCR amplification~~ is 20 ~~mu~~ μL.

Claim 9. (withdrawn) A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for amplification and detection of Cyt b gene contains oligonucleotides with the sequences (SEQ ID NOS:1-2):

CYT1: 5' TGA YTT GAA RAA CCA YCG TTG 3'

CYT2: 5' CTC CAR TCT TCG RYT TAC AAG 3'

Claim 10. (withdrawn) A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for reamplification and detection of Cyt b gene contains oligonucleotides with the sequences (SEQ ID NOS:3 and 2):

CBI-L: 5' CCA TCC AAC ATC TCA GCA TGA TGA AA 3'

CYT2: 5' CTC CAR TCT TCG RYT TAC AAG 3'

Claim 11. (currently amended) A method ~~as claimed in of~~ claim 1<sub>2</sub> wherein the ~~primer set (forward and backward primers)~~ set of forward and backward primers used for PCR amplification and detection of D-Loop gene ~~contains~~ comprises the ~~oligonucleotides with the~~ oligonucleotide sequences:

PRO-L: 5' CTA CC 3'

D-LOOP H: 5' CCT GAA GTA GGA ACC AGA TG 3' (SEQ ID NO:4)<sub>2</sub>

Claim 12. (withdrawn) A method as claimed in claim 1 wherein the forward and backward primers used for PCR amplification of ITS2 gene were

ITS1 F: 5' TTG TAC ACA CCGCCGTCGC 3' (SEQ ID NO: 41)

ITS2 R: 5' ATA TGC TTA AAT TCA GCG GG 3' (SEQ ID NO:6)

Claim 13. (withdrawn) A method as claimed in claim 1 wherein the forward and backward primers used for PCR reamplification of ITS2 gene from ITS1 F and ITS2 R PCR amplification were

ITS2 F: 5' CTA CGC CTG TGT GAG TGT C 3' (SEQ ID NO: 5)

ITS2 R: 5' ATA TGC TTA AAT TCA GCG GG 3' (SEQ ID NO: 6)

Claim 14. (withdrawn) A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of Rhodopsin gene Rod contains oligonucleotides with the sequences (SEQ ID NOS: 8 and 7):

ROD-F: 5' CAT ATG AAT ACC CTC ACT ACT ACC 3'

ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'

Claim 15. (withdrawn) A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 12S RNA gene contains oligonucleotides with the sequences (SEQ ID NOS: 9-10):

12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'

12 SB-H: 5' AGA GTG ACG GGC GGT GTG T 3'

Claim 16. (withdrawn) A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 16S RNA gene contains oligonucleotides with the sequences:

16 SAR-L: 5' CGC CTG TTT ATC AAA AAC AT 3' (SEQ ID NO: 11)

16 SBR-H: 5' CCG GTC TGA ACT GAG ATC ACG T 3' (SEQ ID NO: 12)

Claim 17. (withdrawn) A method as claimed in claim 1 wherein the forward and backward primers used for PCR amplification of Rhodopsin gene Rod were (SEQ ID NOS: 8 and 7):

ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3'

ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3'

Claim 18. (withdrawn) A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 12S RNA gene were (SEQ ID NOS: 9-10):

12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3'

12 SB-H: 5' AGA GTG ACG GGC GGT GTG T 3'

Claim 19. (withdrawn) A method as claimed in claim 1 wherein the primer set (forward and backward primers) used for PCR amplification of 16S RNA gene were (SEQ ID NOS: 11-12):

16 SAR-L: 5' CGC CTG TTT ATC AAA AAC AT 3'

SBR-H: 5' CCG GTC TGA ACT CAG ATC ACG T 3'

Claim 20. (withdrawn) A method claimed in claim 1 wherein the 12S RNA gene and 16S RNA gene in the myctophid fish *Stenobrachius leucopsarus* were amplified by PCR.

Claim 21. (withdrawn) A method claimed in claim 1 wherein the 12S RNA and 16S RNA gene in myctophid fish *Diaphus theta* were eluted by PCR amplification.

Claim 22. (withdrawn) A method claimed in claim 1 wherein the elution of PCR amplification products of myctophid fish *Protomyctophum crockeri*, resulted in 12 S RNA.

Claim 23. (withdrawn) A method claimed in claim 1 wherein the elution of PCR amplification products of myctophid fish *Protomyctophum crockeri*, resulted in 16 S RNA.

Claim 24. (withdrawn) A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Tarletonbeania crenularis*, resulted in 12 S RNA.

Claim 25. (withdrawn) A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Tarletonbeania crenularis*, resulted in 16 S RNA.

Claim 26. (withdrawn) A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Lampanyctus regalis*, resulted in 12 S RNA.

Claim 27. (withdrawn) A method claimed in claim 1 wherein the elution of PCR amplification of myctophid fish *Lampanyctus regalis*, resulted in 16 S RNA.

Claim 28. (currently amended) A method ~~claimed in~~ of claim 1, wherein the ~~cycle-sequencing primer~~ concentration of the primer used for cycle sequencing the eluted DNA is was 2 .mu. 2 µL.

Claim 29. (withdrawn) A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:

CYT 1: 5' TGAYTTGAARAACCAAYCGTTG 3' (SEQ ID NO: 1)

Claim 30. (withdrawn) A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:

CYT 2: 5' CTC CAR TCT TCG RYT TAC AAG 3' (SEQ ID NO: 2)

Claim 31. (withdrawn) A method claimed in claim 1 wherein the cycle sequencing primer for CYT b gene consisted of oligonucleotides with the sequence:

CBI-L: 5' CCA TCC AAC ATC TCA GCA TGA TGA AA 3' (SEQ ID NO: 3)

Claim 32. (currently amended) A method ~~claimed in~~ of claim ~~1~~ 4, wherein the cycle sequencing forward primer for the D-Loop ~~region consisted of oligonucleotides with the gene comprises the oligonucleotide~~ sequence:

PRO-L: 5' CTA CC 3'

Claim 33. (currently amended) A method ~~claimed in~~ of claim ~~1~~ 4, wherein the ~~backward~~ cycle sequencing backward primer for D-Loop ~~region consisted of oligonucleotides with the gene comprises the oligonucleotide~~ sequence:

D-LOOP H: 5' CCT GAA GTA GGA ACC AGA TG 3' (SEQ ID NO:4).

Claim 34. (withdrawn) A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of ITS2 gene consisted of oligonucleotides with the sequence:

ITS 1-F: 5' TTG TAC ACA CCG CCC GTC GC 3' (SEQ ID NO: 41)

Claim 35. (withdrawn) A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of ITS2 gene consisted of oligonucleotides with the sequence:

ITS2-R: 5' ATA TGC TTA AAT TCA GCG GG 3' (SEQ ID NO: 6)

Claim 36. (withdrawn) A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of Rhodopsin gene Rod consisted of oligonucleotides with the sequence:

ROD-F: 5' CAT ATG AAT ACC CTC AGT ACT ACC 3' (SEQ ID NO: 8)

Claim 37. (withdrawn) A method as claimed in claim 1 wherein the backward primer used for cycle sequencing consisted of oligonucleotides with the sequence:

ROD-R: 5' TCT TTC CGC AGC ACA ACG TGG 3' (SEQ ID NO: 7)

Claim 38. (withdrawn) A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of 12S RNA gene consisted of oligonucleotides with the sequence:

12 SA-L: 5' AAA CTG GGA TTA GAT ACC CCA CTA T 3' (SEQ ID NO: 9)

Claim 39. (withdrawn) A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of 12S RNA gene consisted of oligonucleotides with the sequence:

12 SB-H: 5' AGA GTG ACG GGC GGT GTG T 3' (SEQ ID NO: 10)

Claim 40. (withdrawn) A method as claimed in claim 1 wherein the forward primer used for cycle sequencing of 16S RNA gene consisted of oligonucleotides with the sequence:

16 SAR -L: 5' CGC CTG TTT ATC AAA AAC AT 3' (SEQ ID NO: 11)

Claim 41. (withdrawn) A method as claimed in claim 1 wherein the backward primer used for cycle sequencing of 16S RNA gene consisted of oligonucleotides with the sequence:

16 SBR-H: 5' CCG GTC TGA ACT CAG ATC ACG T3' (SEQ ID NO: 12)

Claim 42. (withdrawn) A method as claimed in claim 1 wherein the extension products of 12 S RNA gene region are purified by conventional methods.

Claim 43. (withdrawn) A method as claimed in claim 1 wherein the extension products of 16 S gene region are purified by conventional method.

Claim 44. (withdrawn) A method as claimed in claim 1 wherein the extension products of CYT b gene are purified by conventional method.

Claim 45. (withdrawn) A method as claimed in claim 1 wherein the extension products of ROD gene are purified by conventional method.

Claim 46. (currently amended) A method ~~as claimed in~~ of claim ~~1~~ 4, wherein the extension products of the D-Loop ~~control region~~ gene are purified by conventional method.

Claim 47. (withdrawn) A method as claimed in claim 1 wherein the extension products of ITS2 region are purified by conventional method.

Claim 48. (withdrawn) A method as claimed in claim 1 wherein the extension products of 12 S RNA gene region was sequenced in an automated sequencer.

Claim 49. (withdrawn) A method as claimed in claim 1 wherein the extension products of 16 S gene region was sequenced in an automated sequencer.

Claim 50. (withdrawn) A method as claimed in claim 1 wherein the extension products of CYT b gene was sequenced in an automated sequencer.

Claim 51. (withdrawn) A method as claimed in claim 1 wherein the extension products of ROD gene was sequenced in an automated sequencer.

Claim 52. (currently amended) A method as ~~claimed in of~~ claim ~~1~~ 4, wherein the extension products of D-Loop ~~control-region gene~~ gene ~~was~~ are sequenced in an automated sequencer.

Claim 53. (withdrawn) A method as claimed in claim 1 wherein the extension products of ITS2 region was sequenced in an automated sequencer.

Claim 54. (withdrawn) A method as claimed in claim 1 wherein the identity of the gene 12S RNA is confirmed by Blast Email.

Claim 55. (withdrawn) A method as claimed in claim 1 wherein the identity of the gene 16S RNA is confirmed by Blast Email.

Claim 56. (withdrawn) A method as claimed in claim 1 wherein the identity of the gene CYT b is confirmed by Blast Email.

Claim 57. (withdrawn) A method as claimed in claim 1 wherein the identity of the gene ROD is confirmed by Blast Email.

Claim 58. (currently amended) A method ~~claimed in of~~ claim ~~1~~ 4, wherein the identity of the D-Loop gene is confirmed by Blast email.

Claim 59 (withdrawn) A method as claimed in claim 1 wherein the identity of the gene ITS2 is confirmed by Blast Email.

Claim 60. (currently amended) A method ~~claimed in of~~ claim 1, wherein the cloning vector ~~used for cloning was~~ is Bluescript ~~KS-sup-~~ KS<sup>-</sup> phagemid.

Claim 61. (currently amended) A method ~~claimed in of~~ claim 1, wherein the cloning vector ~~used for cloning had~~ comprises an ampicillin resistance gene for selection.

Claim 62. (currently amended) A method ~~claimed in of~~ claim 1, wherein the cloning vector ~~used for cloning had~~ comprises a Lac Z gene for the selection of blue/white ~~colony selection colonies~~.

Claim 63. (currently amended) A method ~~claimed in of~~ claim ~~1~~ 60, wherein the ~~Col E 1 was the~~ origin ~~for of~~ replication of the KS<sup>-</sup> phagemid in the absence of a helper phage is Col E1.



Claim 64. (currently amended) A method ~~claimed in of~~ claim 1, wherein the cloning vector further comprises an F 1 (-) origin for recovery of antisense strand of lac Z gene when a host strain containing contains the bluescript II phagemid.

Claim 65. (currently amended) A method ~~claimed in of~~ claim 1, wherein the host cells used for electro-transformation ~~were~~ are *E. coli* blue bacteria (~~Bacteria Strain XL 1 blue~~) XL1-Blue: F':::Tn10, pro A<sup>+</sup> sup. + B<sup>+</sup> sup. + lacI<sup>sup.g</sup> (lacZ)M15/recA1 endA1 gyrA9-6(Nal<sup>sup.r</sup>) thi hsdR17(r<sup>+</sup> sub.k<sup>sup.</sup> m<sup>sup.</sup> sub.k<sup>sup.</sup> +) supE44 relA1 lac Bacteria Strain XL1-Blue: F':::Tn10, pro A<sup>+</sup> B<sup>+</sup> lacI<sup>q</sup> (lacZ)M15/recA1 endA1 gyrA96(Na1') thi hsdR17(r<sup>+</sup> m<sup>+</sup>) supE44 relA1 lac.

Claim 66. (currently amended) A method ~~claimed in of~~ claim 1, wherein the ~~probes are containing oligonucleotide sequences are cloned~~ DNA probe is selected from a group of genes consisting of Cyt b, D-Loop, ITS2 and Rod genes.

Claim 67. (withdrawn) A method as claimed in claim 1 wherein the probes of CYT b gene is an oligonucleotide sequence named as PSL CYTL.

Claim 68. (withdrawn) A method as claimed in claim 1 wherein the probes of ITS 2 gene is an oligonucleotide sequence named as PSL ITS 2F.

Claim 69. (currently amended) A method ~~claimed in of~~ claim 1 ~~66~~, wherein the ~~probes of DNA probe for the D-Loop control region gene is an oligonucleotide sequence named as~~ PSL PROL.

Claim 70 (withdrawn) A method as claimed in claim 1 wherein the PCR amplified sequence of ROD gene probe is named as ROD SLMB.

Claim 71. (currently amended) A method ~~claimed in of~~ claim 1 ~~69~~, wherein the ~~PCR~~ amplified sequence of the DNA probe for the D-Loop gene ~~probe~~ is ~~named as~~ D-Loop SLMB.

Claim 72. (withdrawn) A method as claimed in claim 1 wherein the PCR amplified sequence of ITS 2 gene probe is named as ITS 2 SLMB.

Claim 73. (withdrawn) A method as claimed in claim 1 wherein the PCR amplified sequence of Cyt b gene probe is named as Cyt L SLMB.

Claim 74. (withdrawn) The nucleotide base sequences of PSL CYTL (747 bp) comprising (SEQ ID NO: 42):

5' CTTNCCCATT TTGGGCGCTT NGGCNCGCTN CTCCNCGAGA CTCTGCGTAN  
TAATCCAANT CNCTNCGGGC CNCTCCCTAC CANTNCNCTA CACCNCAAAT

TNCAACCCNG TTTCCTCATC ANTCAACCAC ATCTGTCGAA AACNTCAACT  
ACGGCTGACT AATCCGAAAA CATGCACGCT AACGGTGCCT CTTTCTTCTT  
CATCTGTATT TATCTNCNCN TTGGANGAGG ACTATNCTAC GGATCCTACC  
TCTACGAAGA GACGTGAGGT GTTGGTGTTA TTCTTCTCCT TCTAATAATG  
ATGACTGCNT TTGTTGGCTA TGTGCTNCCC NGAGGACAAA TGTCCTTTTG  
AGGTGCTACT GTCATTACAA NCCTACTCTC TGCTGTNCCG TNTGTTNGCG  
GCNCTCTANT TCAATGAATT TGAGGTGGCT TCTCCGTAAA CACGCAACGC  
TCACTCGTTT CTTCGCNTTC CACTTCTTGT TCCCATTGTG TGTCGCNGCT  
ATAACCNNGG TTCACCNGAT TTNCCGACAT CAAACAGGCT CTAAANCCCC  
CCCGGNTTGA CTCCATACAA CAAAACCCTC CACCCTATTC NCTATAAAAC  
TCTAGGTTTCG TGCCCGTATT GGCTTACTTC ATGNCTATTT CCCNGNCGGA  
GGGACNAAAA TTCCTGCACC CCCTCCCCNC AAAATAAANA ATGTGTCTNT  
CCTACCANAA AACAACNNAN ACGGGGTNTG CNCTTCCATC ATCCACN 3'

Claim 75. (withdrawn) The nucleotide base sequences of PSLITS2F comprises:  
(225 BP) (SEQ ID NO: 43)

5' TCTACGATCT ACCGGCNTTT NNTGTGGAAA GACGATCATG CATTTATGTG  
TGTCTTTCTA TGGATTTGAA CCGTGTGGTA CGTCTTTGCG TACTGCTTGG  
AAGGCTCAAC TTGCTTCTGT CCTTCTCTTG CAGTCTCGCA CTGTCTATGC  
AACGTGTTCT ACTTCGACTT CTGTGCAAAA ATCTTACTTT TGACCTCAGA  
TCAGACAAGA CTACCCGCTG AATTT 3'

Claim 76. (currently amended) A method of claim 69, wherein the The  
nucleotide base ~~sequences~~ sequence of PSL PROL comprises: (750 BP) (SEQ ID  
NO:44)

5' CCTTTTCGGN ATAGGCCCAN CTCAAATGAA TTCCTTCTCT CCTGGTCCAA  
GCCCAAACCTG TGGACGGCAG GTTGACAATG GTTACAAATC GTGACAAATC  
GGCTACATAA TTGCCGATAG CGATGTCGTC AAACCAAGTC AAACAATGGC  
CGATGTATAT CGGCCAAACC CATATATGGG TCTGGCTGTA GTTTGTGTTG  
AGCAACGTCA CACCAAGTGC TGGTCAGCAT ATAAGATGTT GACATCTTGC  
AACATCTTAC CCACAGACAG ACAGTTACGG CTGCTTACGA ANGGCGCTAG  
TGTTGTGGTG AGAAACGAAG ATACATACGT CAAACAGACG CCGGTGCACT  
TGAAGACACT GTTTGAAGGT GCCGCACTAC TTGACAGACA GCCCATGATG  
CGCTGGACAG TGACCAAAGC TACNGGAGGA CCANATGGAA ATCCTGTTGG

CGTTGCCGTG GGA CTCAAGT TGTACACTTT TGGATGGTTG ATCACTANAN  
CCGCTGCCGG GAGAAGCACT CGCTCCTGGT TCACTAATCA GATTGAGGTT  
AACCANATTG ANGTAACAT CTTCAACACA GTGTCTTTAT GCTGGATGAA  
ATTNAGCCCA CNGGACACCA NAAAAGAATT NCCNCTGGTT CTNNCGGGGG  
NCCCCNNNAA CGNNTNTTCC CTTNTCTCN NNNGCGGNGA AGTTNCCCCC  
CCCCACTNAN NTCTTCCTTC AANANNTTTC CNCCNNNAGA GGTTTTCCCN 3'

Claim 77. (withdrawn) The nucleotide base sequences of ROD PSL SLMB comprises: (748 BP) (SEQ ID NO: 45)

5' CCTGGTAGGG TTCCCCGTCA ACTTCCTCAC ACTGTACCTC ACNTTCGAGC  
ACAAGAAGCT ACTAACCCCC TTAAGTACA TCCTGCTCAA CCTGGCGGTC  
GGAGACCTCC TGATGGTGTA AGGAGGGTTC ACCACCACCA TCTACACCTC  
CATGCACGGC TACTTCGTCC TAGGGAACT GGGCTGCGCC ATCGAAGGTT  
TCATGGCCAC CCATGGTGGT CAGGTCGCCC TTTGGTCCCT GGTGGTTTTG  
GCCGTGGAAA GGTGGCTGGT CGTCTGCAAN CCCATCTCCA GCTTCCGCTT  
CCAGGAGTCC CACTCCCTCA TGGGCCTGGC CGTGACCTGG GTGATGGCGA  
CGGCTTGTTT TGTGCCCCC CTGGGTCGGC TGGTCTCGCT ACATCCCAGA  
AGGCATGCAG TGCTCATGCG GAATGGACTA CTAACTCCC GCGCCGGGCG  
TCAACAATGA ATCCTACGTN GTGTACATGT TCNTCANAAA AANAATNGGA  
CCNCNGGGCG ATCATNTTGN TANGNNAAGG CCAGNTGNTG NGAGCAGTCA  
AGGCGGCCGC CGCCGCCCAG CAAGAGTCCG AGACCACCCA GAGGGCCGAG  
AGGGAAGTCA CCCGNATGGT NATNANGATG GTNATNTCNT TCNTGGTAAG  
NAGGGNGCCA NACGCCAGCG TGGCCTGGTG GATCTTNNGN AACCAGGGNG  
CAGAATTAGG CCCNGTNTTC ATGACCCTGC CGGCNTTCTT TGCCAAGA 3'

Claim 78. (withdrawn) A method as claimed in claim 1 wherein FORWARD (L) primers of CYT b gene region for myctophid fish *Stenobrachius leucopsarus* is an oligonucleotide comprising (SEQ ID NO: 18):

5' CAA CCT CAT CTG TCG TAA AC 3'

and having the following characteristics:

- i. is a 20-mer DNA oligonucleotide (sense),
- ii. has melting temperature of 56.4 degree celius,
- iii. has a molecular weight of 6101.0,
- iv. has no hairpin loops,

- v. has no single dimers,
- vi. has no other dimers,
- vii. has no single bulge loops or internal loops, and
- viii. has no palindromes.

Claim 79. (withdrawn) A method as claimed in claim 1 wherein BACKWARD (H) primer of CYT b gene region for myctophid fish *Stenobranchius leucopsarus* (SLMB) is an oligonucleotide comprising (SEQ ID NO: 17):

5' GCT CGG GCT GCT GGA ATC TT 3'

and having the following characteristics:

- i. is a 20-mer DNA
- ii. is an antisense oligonucleotide
- iii. has a melting point of 70.8 degree celcius.
- iv. has a molecular weight of 6220.1.
- v. has no hairpin loops, no single bulge loops, no other internal loops, no single internal loops, no other bulge loops or palindromes.
- vi. no single dimers or other dimers.

Claim 80. (withdrawn) A method as claimed in claim 1 wherein forward primer of ITS2 F gene region for myctophid fish *Stenobranchius leucopsarus* (SLMB) is an oligonucleotide comprising (SEQ ID NO: 20):

5' ACT TGA CTG ACC TTC TTA CT 3'

and having the following characteristics:

- i. is a 20-mer sense oligonucleotide,
- ii. has a melting point of 51.3 degree celcius,
- iii. has a molecular weight of 6098.0,
- iv. has no palindromes, loops and dimers,

Claim 81. (withdrawn) A method as claimed in claim 1 wherein forward primer of ITS2H gene region for myctophid fish *Stenobranchius leucopsarus* (SLMB) is an oligonucleotide comprising (SEQ ID NO: 19):

5' ATACTCTGCGGACATACTTGACTG 3'

and having the following characteristics:

- i. is a 24-mer antisense oligonucleotide,
- ii. has a melting point of 65.4 degree celcius.

- iii. has a molecular weight of 7407.9.
- iv. has no palindromes, loops and dimers.

Claim 82. (currently amended) A method ~~claimed in of~~ claim 1 32, wherein the forward primer ~~of~~ for the PRO-L gene for of myctophid fish *Stenobranchius leucopsarus* (SLMB) is an oligonucleotide comprising (SEQ ID NO:21):

5' CAG TCT CGT CAA ACC AAG TCA AAC 3'

and having the following characteristics:

- i. is a 24-mer sense oligonucleotide,
- ii. has a melting point of 67.8 degree Celcius,
- iii. has a molecular weight of 7354.9, and
- iv. has no palindromes, loops and dimers.

Claim 83. (currently amended) A method ~~claimed in of~~ claim 1 33, wherein the backward primer for the D loop ~~for mitochondrial control region (dloop H)~~ gene region ~~for of~~ myctophid fish *Stenobranchius leucopsarus* is an oligonucleotide comprising (SEQ ID NO:22):

5' ATA ATC ATC CAG CAT AAA CAC AC 3'

and having the following characteristics:

- i. is a 23-mer antisense oligonucleotide,
- ii. has a melting point of 61.2 degree celcius,
- iii. has a molecular weight of 7033.7, and
- iv. has no palindromes, loops and dimers.

Claim 84. (withdrawn) A method as claimed in claim 1 wherein the FORWARD primer (ROD-L) for Rhodopsin gene region of myctophid fish *Stenobranchius leucopsarus* is an oligonucleotide comprising (SEQ ID NO: 23):

5' CCT GGT AGA GTT CGC CGT CA 3'

and having the following characteristics:

- i. is a 20-mer sense oligonucleotide
- ii. has a melting point of 67.4 degree celcius.
- iii. has a molecular weight of 6189.0.
- iv. has no palindromes, loops and dimers.

Claim 85. (withdrawn) A method as claimed in claim 1 wherein the backward primer (ROD-H) for Rhodopsin gene region of myctophid fish *Stenobrachius leucopsarus* is an oligonucleotide comprising (SEQ ID NO: 24):

5' CGT GTT CCT TAT CAT TGT GCC T 3'

and having the following characteristics:

- i. is a 22-mer antisense oligonucleotide
- ii. has a melting point of 66.4 degree celcius.
- iii. has a molecular weight of 6738.4.
- iv. has no palindromes, loops and dimers.

Claim 86. (previously presented) A method as claimed in claim 1 wherein the forward primer of 16S-L of the myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising (SEQ ID NO: 26):

5' CAC CAG CCA AGT ATG TTT CTC 3'

and having the following characteristics:

- i. is a 21-mer sense oligonucleotide
- ii. has a melting point of 61.5 degree celcius.
- iii. has a molecular weight of 6421.4.
- iv. has no palindromes, loops and dimers.

Claim 87. (previously presented) A method as claimed in claim 1 wherein the backward primer of 16s rRNA of myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising (SEQ ID NO: 25):

5' TCG TAG TTC AGC AGT CAG 3'

and having the following characteristics:

- i. is a 18-mer antisense oligonucleotide
- ii. has a melting point of 51.2 degree celcius.
- iii. has a molecular weight of 5594.7.
- iv. has no palindromes, hairpin loops and dimers.

Claim 88. (previously presented) A method as claimed in claim 1 wherein the forward primer 16S-L of myctophid fish *Lampanyctus regalis* is an oligonucleotide comprising (SEQ ID NO: 28):

5' CTA TTC GCC TCG CTC AGA C 3'

and having the following characteristics:

- i. is a 19-mer sense oligonucleotide
- ii. has a melting point of 62.1 degree celcius.
- iii. has a molecular weight of 5779.8.
- iv. has no palindromes, hairpin loops and dimers.

Claim 89. (previously presented) A method as claimed in claim 1 wherein a primer 12S-H for *Lampanyctus regalis* (LRMB) is an oligonucleotide comprising (SEQ ID NO: 27):

5' GCC TCC ATC ATC CCT CAC CTT AC 3'

and having the following characteristics:

- i. is a 23-mer antisense oligonucleotide
- ii. has a melting point of 70.8 degree celcius.
- iii. has a molecular weight of 6895.5
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 90. (previously presented) A method as claimed in claim 1 wherein the primer 12S-L for *Lampanyctus regalis* (LRMB) is an oligonucleotide comprising (SEQ ID NO: 28):

5' CTA TTC GCC TCG CTC AGA C 3'

and having the following characteristics:

- i. is a 19-mer sense oligonucleotide
- ii. has a melting point of 62.1 degree celcius.
- iii. has a molecular weight of 5779.8
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 91. (previously presented) A method as claimed in claim 1 wherein 16S-L forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising (SEQ ID NO: 30):

5' AAA TCC GCC CTT ATG TGT GTT C 3'

and having the following characteristics:

- i. is a 22-mer sense oligonucleotide
- ii. has a melting point of 67.9 degree celcius.
- iii. has a molecular weight of 6756.4

iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 92. (previously presented) A method as claimed in claim 1 wherein 16S-H backward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising (SEQ ID NO: 29):

5' CTC CGT CCG TCT CGC CTC TG 3'

and having the following characteristics:

- i. is a 20-mer antisense oligonucleotide
- ii. has a melting point of 71.7 degree celcius.
- iii. has a molecular weight of 6052.0
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 93. (previously presented) A method as claimed in claim 1 wherein 12S-H forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising (SEQ ID NO: 31):

5' CAT CGG CTT GCT CTA TTC CTT G 3'

and having the following characteristics:

- i. is a 22-mer antisense oligonucleotide
- ii. has a melting point of 68.8 degree celcius.
- iii. has a molecular weight of 6723.4
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 94. (previously presented) A method as claimed in claim 1 wherein 12S-L forward primer for *Diaphus theta* (DTMB) is an oligonucleotide comprising (SEQ ID NO: 32):

5' TCT ATC GGC GGC GTA TCA C 3'

and having the following characteristics:

- i. is a 19-mer sense oligonucleotide
- ii. has a melting point of 65.8 degree celcius.
- iii. has a molecular weight of 5859.8
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.



Claim 95. (previously presented) A method as claimed in claim 1 wherein 16S-H primer for *Tarletonbeania crenularis* ( TCMB) is an oligonucleotide comprising (SEQ ID NO: 33):

5' GGC GAT TCT ACG GCA CGG GCG 3'

and having the following characteristics:

- i. is a 21-mer antisense oligonucleotide
- ii. has a melting point of 80.4 degree celcius.
- iii. has a molecular weight of 6568.3
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of

either single or other kinds.

Claim 96. (previously presented) A method as claimed in claim 1 wherein 16S-L forward primer for *Tarletonbeania crenularis* ( TCMB) is an oligonucleotide comprising (SEQ ID NO: 34):

5' AAA CTG GTC CTC AAC TAT GTC A 3'

and having the following characteristics:

- i. is a 22-mer sense oligonucleotide
- ii. has a melting point of 60.7 degree celcius.
- iii. has a molecular weight of 6758.5
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of

either single or other kinds.

Claim 97. (previously presented) A method as claimed in claim 1 wherein 16S-H backward primer for *Tarletonbeania crenularis* ( TCMB) is an oligonucleotide comprising (SEQ ID NO: 33):

5' GGC GAT TCT ACG GCA CGG GCG 3'

and having the following characteristics:

- i. is a 21-mer antisense oligonucleotide
- ii. has a melting point of 80.4 degree celcius.
- iii. has a molecular weight of 6568.3
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of

either single or other kinds.

Claim 98. (previously presented) A method as claimed in claim 1 wherein 12S-H backward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising (SEQ ID NO: 35):

5' CCG ATT CAG CCA CGA TTC CCT C 3'

and having the following characteristics:

- i. is a 22-mer antisense oligonucleotide
- ii. has a melting point of 74.6 degree celcius.
- iii. has a molecular weight of 6671.4
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 99. (previously presented) A method as claimed in claim 1 wherein 12S-L forward primer for *Tarletonbeania crenularis* (TCMB) is an oligonucleotide comprising (SEQ ID NO: 42):

5' CCT AAA GCC CAG ATA ACT ACA 3'

- i. is a 21-mer sense oligonucleotide
- ii. has a melting point of 59.2 degree celcius.
- iii. has a molecular weight of 6432.3
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 100. (previously presented) A method as claimed in claim 1 wherein 16S-H backward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising (SEQ ID NO: 37):

5' CGT GTT CTG ATG ATG ATG TGC T 3'

- i. is a 22-mer antisense oligonucleotide
- ii. has a melting point of 64.7 degree celcius.
- iii. has a molecular weight of 6867.5
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 101. (previously presented) A method as claimed in claim 1 wherein 16S-L forward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising (SEQ ID NO: 38):

5' ATT CCT TCC TCT TAG TAT G 3'

- i. is a 19-mer sense oligonucleotide
- ii. has a melting point of 49.5 degree celcius.
- iii. has a molecular weight of 5799.8
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 102. (previously presented) A method as claimed in claim 1 wherein 12S-H backward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising (SEQ ID NO: 39):

5' GCT GAA CTT ACT ATG CCC TAC T 3'

- i. is a 22-mer antisense oligonucleotide
- ii. has a melting point of 60.3 degree celcius.
- iii. has a molecular weight of 6725.4
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 103. (previously presented) A method as claimed in claim 1 wherein 12S-L forward primer for *Protomyctophum crockeri* (PCMB) is an oligonucleotide comprising (SEQ ID NO: 40):

5' CCG ATT GAC GCC GAA CTA TG 3'

- i. is a 20-mer sense oligonucleotide
- ii. has a melting point of 68.1 degree celcius.
- iii. has a molecular weight of 6182.1
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 104. (previously presented) A method as claimed in claim 1 wherein 16S-H backward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising (SEQ ID NO: 15):

5' TAC GCA TAA CGG CTC TGG 3'

- i. is a 18-mer DNA oligonucleotide ( Antisense)
- ii. has a melting point of 61.4 degree celcius.
- iii. has a molecular weight of 5579.7
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 105. (previously presented) A method as claimed in claim 1 wherein 16S-L forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising (SEQ ID NO: 16):

5' CTA CTA CAC CTC AAC TAC ATC T 3'

- i. is a 22-mer sense oligonucleotide
- ii. has a melting point of 52.4 degree celcius.
- iii. has a molecular weight of 6638.4
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 106. (previously presented) A method as claimed in claim 1 wherein 12S-H forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising (SEQ ID NO: 13):

5' CCC ACT CAC TGC TAA CTC C 3'

- i. is a 19-mer sense oligonucleotide
- ii. has a melting point of 58.4 degree celcius.
- iii. has a molecular weight of 5708.8
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.

Claim 107. (previously presented) A method as claimed in claim 1 wherein 12S-L forward primer for *Stenobrachius leucopsarus* (SLMB) is an oligonucleotide comprising (SEQ ID NO: 14):

5' GGC TAA CTA CAA TCA TCT GCT 3'

- i. is a 21-mer sense oligonucleotide
- ii. has a melting point of 58.5 degree celcius.
- iii. has a molecular weight of 6445.2
- iv. has no hairpin loops, dimers, palindromes or bulges and internal loops of either single or other kinds.